Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2110

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **3 (5):** 121-127 (2015)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Contamination profile of DDT and HCH in packaged milk samples collected from Haridwar, India

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ABSTRACT

Milk is the source of casein protein, vitamins, calcium and phosphorus and plays vital role in strengthening the human body tissues. Now a days most food items are contaminated by different synthetic chemicals including milk. DDT (Dichlorodiphenyl trichloroethane) and HCH (Hexachlorohexane) come under the organochlorine insecticides group and indiscriminately used in agriculture and in control of mosquitoes in different health programmes in India. These insecticides are lipophilic and got accumulated in fatty tissues of organisms and environment. This investigation was undertaken to evaluate the levels of DDT metabolites and HCH isomers in packaged milk samples collected from Haridwar, Uttarakhand, India. Total 27 milk samples of different brands viz Paras, Amul Gold, Ananda, Dairy Best and Mother Dairy were collected and analysed using Gas Chromatographic Mass Spectrometry. The HCH was not detected in Ananda, Dairy best and Mother Dairy whereas DDT and its metabolites were found in all the brands. The concentration of HCH isomers and DDT metabolites were recorded under the standard limits of WHO (World Health Organization).

Key word: *DDT, HCH, lipophilic, insecticides, WHO*

INTRODUCTION

Milk is the rich source of the proteins, vitamins and other essential elements. It contains casein which is only present in milk and source of many amino acids. Protein is required for the growth and development of human tissues and immunity. The calcium, phosphorus, magnesium, and potassium are the important constituents of milk and it also contains vitamin B_2 as well as vitamins A and D. Milk is at the risk of contamination by a variety of chemicals which persists in environment after disposal or their application. The major chemical contaminants are antibiotics, disinfectants, nitrites and pesticides[1]. The contamination of milk with pesticides can adversely affects the growth and development of humans especially children up to a significant level.

Organochlorine insecticides are chemically synthesized with chlorine and carbon. These can be divided in three classes viz dichlorodiphenylethanes (DDT, DDD, dicofol), chlorinated cyclodienes (aldrin, dieldrin, heptachlor), hexachlorocyclohexanes (BHC)[2].

Cite this article: Negi, R.K. and Rani, S., Contamination profile of DDT and HCH in packaged milk samples collected from Haridwar, India, *Int. J. Pure App. Biosci.* **3 (5):** 121-127 (2015). http://dx.doi.org/10.18782/2320-7051.2110

The use of synthesized pesticides started in 1949 with the application of DDT (Dichloro-diphenyl-trichloroethane) in malaria control and HCH (Hexa-chloro-hexane) to control locust[3,4]. Organochlorine insecticides were used in high quantities worldwide especially in tropical countries like India to control malaria and in agricultural to control various pests. Now days, maximum number of food items we consume are contaminated with different pesticides. The quantification of organochlorines is being carried out due to its health hazardous and bioaccumulation in food chains. DDT, HCH, aldrin and dieldrin were used in agriculture and public health programmes in early 1980s in various countries. Most of countries now put a ban on these pesticides but some of them are still using by the local farmers and health department due to its versatile nature against various pests[5,6]. Endosulfan is being widely used in cotton growing areas, on vegetable farms, and on coffee plantations[7]. DDT and HCH were used in agriculture and in public health to prevent various pests, weeds, and other pathogens to protect the humans from various diseases. Approximately 25,000 mt/year of organochlorines have been used in India in which DDT contributed 40% of the total[8]. Pesticides are developed under strict regulation with the aim of minimal impact on environment and human health but major concerns were raised when these components are detected in components of food chains. India is one the largest manufacturer of different pesticides in Asia and these insecticides are being used in measurable quantities in agriculture and health sector to combat various pests that may affect agricultural production and population[9] (Sarkar *et al.*, 2012). DDT and HCH are ubiquitous contaminants which undergoes biomagnifications process in food chain[10,11,12]. The presence of DDT metabolites and HCH isomers in various components of environment is due to long range transport and persistence. DDT and HCH were used in public health and agricultural in India[13,14,15,16,17,18]. Dairy milk, Bovine and cow milk contaminated with DDT metabolites and HCH isomers residues have been investigated by various workers across the world[19,20,21,22,23,24,25,26,27,28,29,30].
Therefore, the present study was undertaken to investigate the levels of DDT and HCH in packaged milk samples of different brands sold in Haridwar city, Uttarakhand, India to access the contamination profile of these pesticides.

MATERIALS AND METHODS

All the samples were collected and analyzed following the reported methods[18]. The extraction recovery was more than 90% for all HCH isomers (α, β, γ, δ) and DDT metabolites (o,p DDT, p,p DDT, o,p DDE, p,pDDE, p,pDDD and o,pDDD) in spiked milk samples.

Sample collection and Extraction

A total of 27 milk samples of different brands viz. Paras (09), Amul Gold (4), Ananda (6), Dairy Best (5) and Mother dairy (03) were collected from the Haridwar city of Garhwal region of Uttarakhand state in India **(Fig. 1).** All the collected samples were preserved in saturated solution of potassium dichromate containing 1% amyl alcohol. Three replicates of each sample were analysed as per the previously reported methods.

25 ml milk from each samples was taken for extraction of DDT and HCH residues with methanol and sodium oxalate with addition of 10 ml ethyl ether. The contents were shaken for 5 min and 10 ml petroleum ether was added and was shaken for another 5 min. Petroleum ether extract is separated after the centrifuge at 1500 rpm and transferred to separating funnel containing 250 ml distilled water and 20 ml saturated solution of sodium chloride. The petroleum ether portion was passed through an anhydrous sodium sulfate column and dried to obtain fat.

The weighted fat content was dissolved in 10 ml petroleum ether and transferred to a separating funnel containing 50 ml of acetonitrile. The solvent was shaked vigorously for 1 min and the layers were allowed to separate. Acetonitrile layer was transferred to separating funnel containing 50 ml petroleum ether and shaked for 1 minute after which was again extracted twice with 15 ml petroleum ether. The petroleum ether extract was then passed through an anhydrous sodium sulphate column and was evaporated in a vacuum evaporator.

Cleanup of samples

The extracted samples were processed for the removal of impurities by column chromatography with 5 % deactivated alumina and 1.5 cm of anhydrous sodium sulphate. The column was packed tightly and washed by 50 ml n-hexane. The extracts of samples were loaded in the column with 100 ml n-hexane: benzene (1:1 v/v). The solvent was collected and evaporated to dryness by vacuum evaporator and stored till further analysis by GCMS.

Gas Chromatography Mass Spectrometry (GCMS) Analysis

GC MS analysis was performed using a Bruker Gas Chromatograph coupled with Mass Detector model Scion TQ 436 The column used for the analysis was Zebron : phenomenex Phase - ZB -5MS (L= 60m x 1, D= 0.25mm x df = 0.25 id). The initial oven temperature was hold at 90°C for 3 min and then increased at the rate of 15°C/min up to 150°C and then set for 6 minutes. It was again increased at the rate of 5°C up to 280°C and set for 35 minutes. The injection port was in split less mode and temperature was kept 280°C. Ultra pure helium was used as a carrier gas at a flow rate of 1 ml/min. MS detector was operated at 70 eV in EI autoionization mode. The trap temperature, manifold temperature and transfer line temperature were 170^0C, 40^0C and 270^0C, respectively for MS detector.

RESULTS AND DISCUSSION

Milk is an important source of nutrition for human system. It is a food that has an important position in the human diet. The contamination of milk by pesticides could pose a threat to human health. Therefore it should be free from any type of contamination and residue levels must be under recommended limits in order to minimize the harm to human health[31]. A total of 27 packaged milk samples of different brands were collected from Haridwar and analysed for HCH isomers and DDT metabolites. All the samples were found contaminated with residues of these insecticides. The result of sample analysed are presented in **Table 1**. Out of all brands, HCH isomers were only detected in Paras and Amul Gold whereas DDT metabolites were found in all the brands. Among HCH isomers alpha, beta HCH were detected in the milk samples of Paras and Amul Gold and gamma HCH was only reported in Paras whereas HCH isomers were not detected in the milk samples of Dairy Best, Mother dairy and Ananda. Milk samples of all brands were found contaminated with DDT and its metabolites. In DDT metabolites o,p DDE was absent in the samples of all brands except Amul Gold. Alpha HCH was found more as compared to all the isomers of HCH with mean concentration of 0.083 ppb in Paras brand followed by 0.067 ppb in Amul Gold. Total HCH was also found maximum in Paras as compared to Amul Gold. Total DDT was found maximum in the samples of Paras brand with mean concentration of 14.50 ppb followed by 8.51 ppb in Amul Gold, 8.05 ppb in Ananda, 7.14 ppb in Dairy Best and 4.21 ppb in Mother Dairy. Among DDT metabolites p,p DDE contributed with high levels in all the samples followed by p,p DDD, o,p DDT, p,p DDT, o,p DDD and o,p DDE. The similar pattern of HCH isomers and DDT metabolites were reported in whole milk samples of Uttar Pradesh and Madhya Pradesh[27]. In the present study, some changing patterns of HCH isomers and DDT metabolites have been observed. The HCH isomers were found absent in most of the samples whereas o,p DDE was detected only in one sample. The changing pattern of various HCH isomers and DDT metabolites in whole milk samples were also reported[32].

The Maximum Residue Level of total HCH and total DDT should be 0.05 mg/kg for whole milk basis[33]. The contamination profile level of HCH isomers and DDT metabolites in packaged milk samples of present study was found within the prescribed limit and lower than reported concentration by different workers from India and abroad[34,35,28,32,30]. The tendency of contamination is showing the phasing out of DDT and HCH from the study area. The results of present study are compared with the other investigations in the past from across the world in **Table 2.**

Though the application of HCH is ban in all field and DDT can only be used in public health by heath department in places where required, but residues are detected in food contents due to their persistence nature in environment. These residues got accumulated in plants, and weed which are taken up by herbivores and get accumulated in the fatty tissues of the animal, hence still detected in the milk of animals and consumers of milk are at the risk to these compounds without knowing anything[36]. The residue of the DDT and HCH persists for a long times in the environment and its components, however lesser use of these chemicals can eliminate them from all the components of environment[18]. In the present investigation, it could be established that HCH isomers and DDT metabolites in the study area has no potent risk for public health, however the monitoring of these compounds in other components is necessary to evaluate the exposure level of these chemicals in population. This trend is also reflecting the reduction and responsible use of these chemicals in recent years[37].

Fig. 1: Study sites

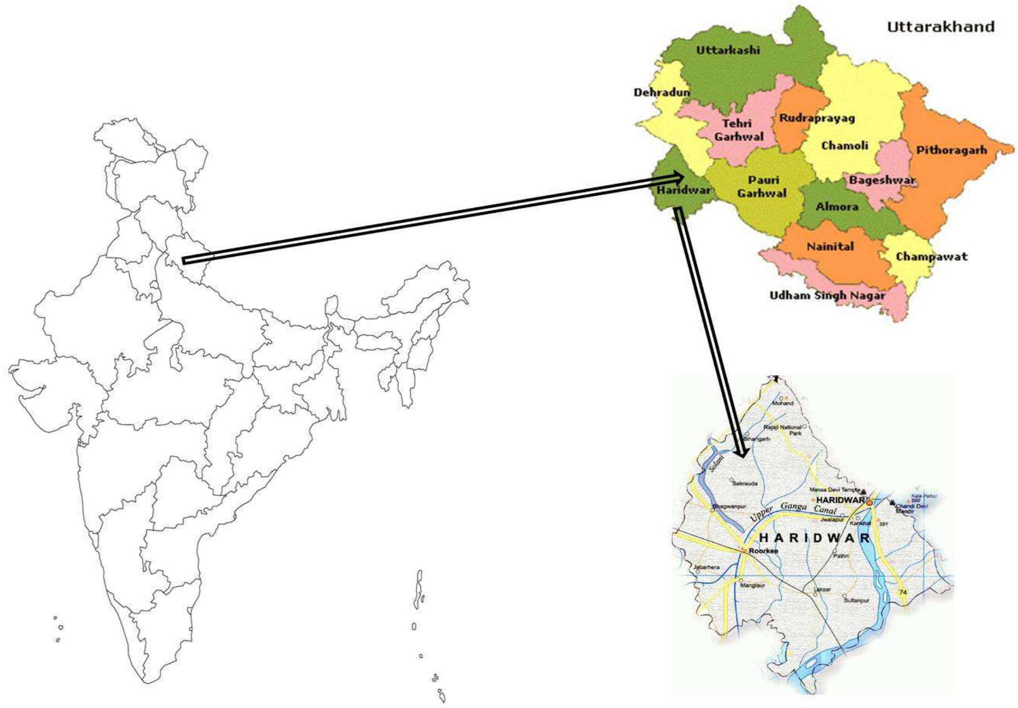

Table 1 : Mean±SE concentration (ppb) of HCH isomers and DDT metabolites in different brands of packaged milk of Haridwar, Uttarakhand

Brand	No. of samples analysed (n)	α-HCH	β-HCH	γ-HCH	δ-HCH	? -HCH	o,p DDT	p,p DDT	o,p DDE	p,p DDE	p,p DDD	o.p DDD	? DDT
Paras	9	0.083±0.01 (ND - 0.75)	0.09±0.00 (ND - 0.76)	0.057±0.038 (ND - 0.26)	ND	0.23±0.09 (ND -0.81)	3.89±1.12 (ND-8.35)	0.61±0.20 (ND - 2.71)	ND	5.95±1.77 (ND -27.77)	3.90±1.20 (ND - 8.38)	0.18±0.09 (ND - 0.67)	14.50±3.12 (ND - 30.12)
Amul Gold	4	0.067± 0.05 (ND -0.27)	0.13±0.02 (ND - 0.52)	ND	ND	0.20±0.03 (ND -0.79)	0.71±0.23 (ND - 2.62)	0.69±0.041 (ND - 2.75)	0.30±0.12 (ND - 1.18)	5.73±1.77 (ND - 20.01)	1.05±0.43 (ND - 2.81)	0.32±0.01 (ND - 0.13)	8.51±2.95 (ND - 29.36)
Ananda	6	ND	ND	ND	ND	ND	2.70 ± 1.20 (ND -6.75)	0.27 ± 0.021 (ND -1.61)	ND	2.16 ± 0.80 (ND - 5.89)	2.90 ± 1.30 (ND - 6.92)	0.034 ± 0.001 (ND - 0.20)	8.05 ± 2.15 (ND - 19.75)
Dairy best	5	ND	ND	ND	ND	ND	0.68±0.06 (ND - 3.40)	0.53±0.04 (ND - 2.49)	ND	3.09±0.72 (0.76 - 5.26)	2.72±1.10 (ND - 5.07)	0.11±0.08 (ND - 0.54)	7.14±1.16 (ND - 10.23)
Mother dairy	3	ND	ND	ND	ND	ND	1.33±0.67 (ND - 3.66)	0.47±0.03 (ND -1.11)	ND	1.09±0.28 (ND -1.60)	1.30±0.78 (ND - 3.65)	0.04±0.001 (ND- 0.13)	4.21±1.34 (1.12 - 8.80)

() - values given in parenthesis are the range;
ND - Not detected

Table: 2 Comparison of present study with contamination reported from other places

Country	References	∑-HCH (mg/kg)	∑-DDT (mg/kg)
China	Zhang (1995)[38]	0.07	
Mexico	Waliszewski *et al*. (1996)[34]	0.098	0.095
India (Delhi)	Mukherjee and Gopal (1993)[39]	0.071	0.057
Slovakia	Prachar *et al*. (1995)[40]	0.015	0.150
India (Haridwar)	Dua et al. (1997)[22]	0.027	0.021
India (all)	Agnihotri (1999)[33]	ND-5.12	0.413
India (Lucknow)	Nigam and Siddiqui (2001)[41]		ND-25.6
India (South)	Surendranath *et al*. (2002)[42]	0.01–0.71	0.015
China	Zhang *et al*. (2003)[43]		ND-0.8
India (North)	Kathpal *et al*. (2004)[44]	0.001–0.209	0.046
India (UP & MP)	Nag and Raikwar (2007)[45]	0.162	0.001–0.649
Present Study (Packaged milk)		ND – 0.0008	ND – 0.029

CONCLUSION

In the present investigation the level of contamination of HCH isomers and DDT metabolites were recorded. HCH was not detected in maximum samples whereas DDT was present in most of the samples but the concentration level was within given limit of WHO. This trend may be due to the responsible use of DDT by the authorities and general population and also indicates the phasing out of these chemicals from the region in animal milk samples but still there is a requirement of further investigation on the residue levels in other components of environments for the sake of human health.

Acknowledgement

Authors are very thankful to University Grant Commission for the BSR research fellowship vides UGC Reference N0. F. 7- 70/2007(BSR) to provide fellowship and Department of Zoology and Environmental Science, Gurukul Kangri University, Haridwar to offer support for the research work.

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